Factor V Activation and Inactivation by Venom Proteases

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Abstract
Blood coagulation factor V is a single-chain glycoprotein with Mr = 330,000 which plays an important role in the procoagulant and anticoagulant pathways. Thrombin activates factor V into factor Va, a two-chain molecule which is composed of a heavy (Mr = 105,000) and a light chain (Mr = 71,000/74,000). Factor Va accelerates factor Xa-catalysed prothrombin activation more than 1,000-fold and under physiological conditions the cofactor activity of factor Va in prothrombin activation is down-regulated by activated protein C. Factor V can also be activated by a wide variety of snake venoms (e.g. from Vipera species, Naja naja oxiana, Bothrops atrox) and by proteases present in the bristles of a South American caterpillar (Lonomia achelous). Some venoms, notably of Vipera lebetina turanica and Lonomia achelous, contain proteases that are able to inactivate factor V or factor Va. Venom factor V activators are excellent tools in studying the structure-function relationship of factor V(a) and they are also used in diagnostic tests for quantification of plasma factor V levels and for the screening of defects in the protein C pathway. In this review, the structural and functional properties of animal venom factor V activators and inactivators is described.

Introduction

For almost every clotting factor in the coagulation cascade there is a venom protease that can activate it. This paper focuses on ani-
mal venom components that can activate or inactivate blood coagulation factor V, a clotting factor which, considering its central position in the coagulation cascade, has a key function in haemostasis.

Factor V is a multi-functional protein which plays an important role in both the procoagulant and anticoagulant pathways [for reviews, see ref. 1, 2]. Factor V (fig. 1) circulates in plasma as a single-chain glycoprotein with a molecular mass of 330 kD. However, factor V is not active as a coagulation factor and in order to express procoagulant activity it has to be first activated by thrombin or factor Xa. Activation proceeds via specific cleavage of peptide bonds at positions 709, 1018 and 1545, and yields factor Va, a heterodimer consisting of a heavy chain of 105 kD and a light-chain doublet of about 71/74 kD.

Factor Va acts as cofactor in factor Xa-catalysed prothrombin activation and it enhances the rate of thrombin formation more than 1,000-fold. Kinetic analysis has shown that factor Va accelerates prothrombin activation by: (1) acting as a receptor that promotes the binding of factor Xa and prothrombin to procoagulant membranes and (2) by enhancing the catalytic activity of factor Xa [2]. Factor V also plays a role in the anticoagulant pathway. Its cofactor activity in prothrombin activation is down-regulated by activated protein C and finally it has been shown that factor V acts as cofactor in activated protein C (APC)-mediated factor VIII inactivation [1, 2].

Animal venom proteins have been used as a tool in investigations of the structure-function relationship of factor V and in the development of diagnostic tests that probe the properties of factor V in both the pro- and anticoagulant pathways.

Animal Venom Factor V Activators and Inactivators

Table 1 summarises the animal venoms which have been reported to contain factor V activators. The venoms of Viperidae are historically linked with blood coagulation. Already around 1960 it was reported that Russell’s viper venom contains both a potent factor X [3] and a factor V activator [4]. In 1978 it was shown that thrombocytin, the throm-
bin-like enzyme from *Bothrops atrox* was able to activate factor V [5] and in the early 1990s our laboratory in Maastricht reported that the venom of several *Naja* species contained a factor V activator [6]. Recently, it was shown that the bristles from certain South American caterpillars contain two different proteases that can activate and inactivate factor V, respectively [7]. The venom factor V activator from *Naja naja oxiana* partially inactivates thrombin-activated factor V [6], and unpublished observations reported in the present paper indicate that the venom from *Vipera lebetina turanica* is also able to inactivate factor Va. In this paper we will provide further information on the structural and functional properties of the factor V activators and inactivators.

**Factor V Activators in Venoms from Viperidae**

Russel's viper venom contains a powerful factor V activator, which is called RVV-V. The ability to activate factor V was first recognized by Hjort [4] in 1957 and RVV-V was purified to homogeneity by Kisiel [8]. RVV-V is a single-chain serine protease which consists of 236 amino acids [9]. This enzyme activates factor V [10, 11] by a specific cleavage of a single peptide bond at Arg1245 [12] which distinguishes it from thrombin which also cleaves factor V at positions 709 and 1018. The product obtained after activation by RVV-V does however have the same procoagulant activity as thrombin-activated factor V [10, 11]. RVV-V is unique in that no other substrate has as yet been identified and it is widely used in research in the field of haemostasis and thrombosis.

The RVV-V purified by Kisiel [8] appeared to contain 3 isoproteins that could be separated by HPLC [9]. The three isoproteins called RVV-Vα, RVV-Vβ and RVV-Vγ, which are present in the purified preparation in a weight ratio 2:1:6, have molecular masses of 29, 27.5 and 29 kD, respectively [9]. The amino acid composition of RVV-Vβ differed from that of RVV-Vα and RVV-Vγ. Since only limited amounts of protein were available, RVV-Vβ was not further characterised. The complete amino acid sequences of RVV-Vα and RVV-Vγ were determined and both contained 236 amino acids and they differed in 6 amino acid residues [9]. It is not known whether RVV-Vα, RVV-Vβ and RVV-Vγ exhibit the same activity in factor V activation since it was not possible to study their functional properties due to the fact that they were only separable by HPLC under denaturing conditions.

Other *Vipera* species also contain factor V activators. Siigur et al. [13, 14] characterised the activator from *Vipera lebetina*. In our laboratory [unpubl. obs.] we compared the factor V activators from *Vipera russelli*, *V. lebetina* and *V. ursini* called RVV-V, LVV-V and

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**Table 1. Animal venom factor V activators**

<table>
<thead>
<tr>
<th>Venom source</th>
<th>Species</th>
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<tr>
<td>Bristles of larvae (caterpillar) of Lepidoptera</td>
<td><em>L. achelous</em></td>
</tr>
<tr>
<td>Venoms of Crotalidae</td>
<td><em>B. atrox</em> (thrombocytin)</td>
</tr>
<tr>
<td>Venoms of Elapidae</td>
<td><em>N. n. oxiana</em></td>
</tr>
<tr>
<td>Venoms of Viperidae</td>
<td><em>V. russelli</em>, <em>V. lebetina</em>, <em>V. ursini</em></td>
</tr>
</tbody>
</table>
Fig. 2. SDS-PAGE analysis of factor V activators purified from the venoms of *V. russelli* (RVV), *V. lebetina* (LVV) and *V. ursini* (UVV). Molecular weight markers are indicated Mr.

Fig. 3. a, b Time courses of activation and inactivation of factor V by vipera activators. a Concentrations of activators were: ○ = 10 nM RVV-V, ▲ = 10 nM LVV-V, ▲ = 9 nM UVV-V and □ = 0.25 nM human α-thrombin. b Inactivation of factor Va by 250 nM LVV-V.

The three venom activators were equally active in activating factor V (fig. 3a). For comparison, the time course of factor V activation by thrombin is also given in this figure. It should be emphasised that the thrombin concentration in the activation mixture was some 40-fold lower than the concentration of the venom activators. This means that thrombin is about 40 times more effective in activating factor V than the *Vipera* activators.

Finally, it was observed that the purified activator from *V. lebetina* (LVV-V) not only activated factor V, but also inactivated factor Va. When thrombin- or venom-activated factor V was incubated with 250 nM LVV-V, the cofactor activity of factor Va in prothrombin activation was lost with a half-life of about 10 min (fig. 3b). Since the concentration of LVV-V used in this experiment was about 25 times higher than in the activation experiments, this means that under normal activa-
tion conditions there is only a minor degra-
dation of factor Va by LVV-V. Currently, we are
investigating which peptide bond cleavages in
factor Va are responsible for LVV-V-cata-
ysed factor Va inactivation.

**Factor V Activator from the Venom of *Naja naja oxiana***

During a screening of the pro- and antico-
agulant properties of snake venoms from the
Elapidae family it was observed that the
venoms from several *Naja* species were able
to activate factor V [6, 15]. The factor V activ-
ator present in the venom of *N. n. oxiana*,
which contained the largest amount of activa-
tor, was purified by repeated chromatography
on a Mono S column [6]. The purified activa-
tor appears to be a single-chain protein which,
as judged by polyacrylamide gel electrophore-
sis in the presence of SDS, has an apparent
molecular mass of 48 kD.

The *N. n. oxiana* protease activates factor
V by cleaving two peptide bonds yielding a
factor Va molecule which, compared with
thrombin-activated factor V, has a somewhat
smaller heavy and larger light chain [6]. These
small differences have a rather large effect on
the cofactor activity of factor Va in prothrom-
bin activation. *N. n. oxiana*-activated factor V
had an 8-fold lower cofactor activity than
thrombin-activated factor V. Addition of *N.
n. oxiana* protease to thrombin activated-fac-
tor V resulted in some 90% loss of the cofactor
activity of factor Va in prothrombin activa-
tion, indicating that the factor V activator
from *N. n. oxiana* cleaves a peptide bond in
both factor V and factor Va that results in the
formation of a factor Va derivative which has
a strongly diminished cofactor activity. SDS-
PAGE in combination with HPLC and amino
cid sequencing of a peptide isolated from a
venom-treated factor Va preparation indi-
cated that the venom activator removed the
last 27 amino acids from the carboxy terminal
domain of the heavy chain of factor Va [16].
This indicates that the venom protease from
*Naja oxiana* cleaves the His^{682}-Asp^{683} peptide
bond in factor V. Kinetic analysis showed that
*Naja oxiana*-activated factor V had a dimin-
ished affinity for factor Xa and prothrombin
[16], which indicates that the Asp^{683}-Arg^{709}
domain of factor Va is essential for optimal
interaction with prothrombin and factor Xa.

**Factor V Activators Present in the Venoms from *Bothrops atrox* and *Lonomia achelous***

Thrombocytin, a protease isolated from the
venom of *Bothrops atrox* by Niewiarowski
et al. [17] and Kirby et al. [18], is also able
to activate factor V [5]. Purified thrombocytin is
a glycoprotein with a molecular mass of 36 kD
which is a thrombin-like serine protease
which not only activates factor V, but also
platelets, factor XIII and factor VIII [17].

Activation of factor V by thrombocytin
proceeds via the cleavage of two peptide
bonds yielding a product which on the basis of
SDS-PAGE is similar to thrombin-activated
factor V [5]. The exact position of the peptide
bonds in factor V that are cleaved by throm-
boctin during activation is not yet known
and there is also no information on the co-
factor activity of thrombocytin-activated fac-
tor V.

It is well known that contact with caterpil-
lars of moths belonging to the genus *Lonomia*
may cause a haemorrhagic syndrome that
likely results from the presence of proteolytic
proteins in the bristles of these caterpillars
which affect coagulation and fibrinolysis [19–
21]. Lopez and Arocha-Pinango [7] identified
a factor V activator in the hemolymph of *L.
achelous*. The partially purified activator ap-

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**Venom Factor V Activators and Inactivators**

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pears to be a thermostable metalloprotease which has a molecular mass less than 97 kD. The crude venom also contains a high molecular mass serine or cysteine protease that inactivates factor V. Unfortunately, there is as yet no information on the peptide bonds that are cleaved in factor V during activation and inactivation by the proteases present in the venom of L. achelous.

References